Annual variation of DNA fragmentation assessed by SCSA™ in equine sperm

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Annual variation of DNA fragmentation assessed by SCSA™ in equine sperm

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The horse is a long-day breeder and seasonal variation in routine semen parameters are well documented in the literature (Jasko et al., Theriogenology 1991; 35: 317-327 / Janett et al., Theriogenology 2003; 60: 453-461). With the establishment of flow cytometry in spermatology, new fertility-related sperm parameters could be identified. The sperm chromatin structure assay (SCSA™) determines sperm chromatin stability based upon flow cytometry after acid denaturation and staining with acridine orange (Evenson et al., Theriogenology 1994; 41: 637-651). Love and Kenney (Theriogenology 1998; 50: 955-972) demonstrated that the integrity and structural stability of equine sperm is related to fertility. Blottner et al. (Animal Reproduction Science 2001; 65: 75-88) compared sperm DNA fragmentation in ejaculates collected in May (breeding-season) and in December (non-breeding season) and found a slightly enhanced susceptibility of sperm DNA to denaturation in December. However, there are no published reports on annual variation of equine sperm DNA integrity, therefore, the aim of this study was to evaluate sperm DNA fragmentation during one year in stallions. Experiments were performed using 10 Warmblood stallions and ejaculates collected every other week from January to December. Routine raw semen parameters (volume, concentration, total sperm count, sperm motility and morphology) were assessed and ejaculates cryopreserved. SCSA™ was performed in frozen-thawed semen samples according to the standardized protocol and the percentages of sperm showing a high DNA Fragmentation Index (% DFI) were calculated (Evenson et al., Theriogenology 1994; 41: 637-651). Data were analyzed using ANOVA to assess the effects of stallion and month of semen collection on % DFI. Correlations between variables were calculated and tested for significance (Fisher's r to z test). Results show that variability of % DFI was high between and within stallions (P<0.05) but **no seasonal pattern (P>.05) was apparent**. Median % DFI varied between stallions from 4.6% (Stallion A) to 31.7% (Stallion F) and the range (Max.-Min.) within stallion varied between 12.4% (Stallion G) and 28.6% (Stallion D) (Fig. 1). Correlations between % DFI and raw semen characteristics were not significant for sperm concentration (*r*=-0.106, P=0.1037), low for ejaculate volume (*r*=0.223, P=0.0005) and total sperm count (*r*=0.198, P=0.0089) but moderate for morphologically normal sperm cells *(r*=-0.537, P=<0.0001) as well as for sperm motility (*r*=-0.575, P=<0.0001). In conclusion a regular assessment of sperm DNA fragmentation is recommended in stallions. Variation within stallions is high and independent of the season. Correlations between % DFI and routinely evaluated sperm parameters such as sperm motility and sperm morphology are only moderate.

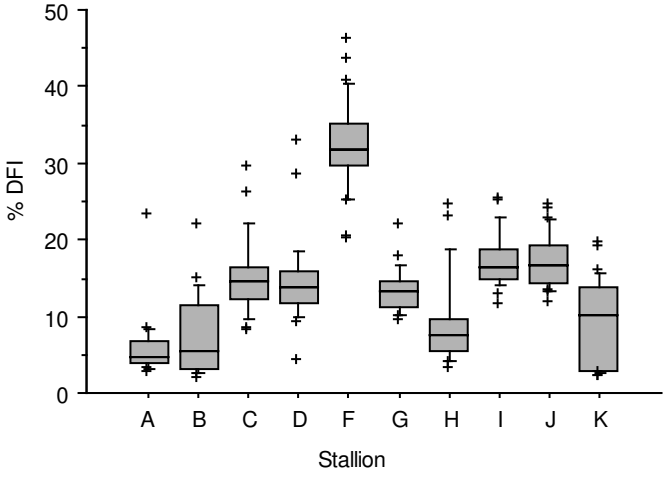

Fig. 1. Box plot showing the DNA Fragmentation Index (% DFI) of sperm collected every other week during one year from 10 stallions.